Studies on Active Ingredients of *Punica granatum* and *Mimosa hamata* Plant Extracts and Their Antimicrobial Activity

A Synopsis

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Introduction

The uses of traditional medicinal plants for primary health care have steadily increased worldwide in recent years. Nature has been a good source of medicinal agents for thousands of years and an impressive number of modern drugs that have been isolated from natural sources (Pannell, 1992). Plants have always been the principal form of medicine in India and presently they are becoming popular throughout the world, as people strive to stay healthy in the face of chronic stress and pollution and to treat illness with medicines that work in count with the body's own defense. There is a wide spread belief that green medicines are healthier and safer or harmless than synthetic one. According to the report of the World Health Organization (WHO), 80% of the world's populations rely mainly on the traditional therapies which involve the use of plant extracts or their active substance. Globally, more than 400,000 species of tropical flowering plants have medicinal properties and this has made traditional medicine cheaper than modern medicine (Odugbemi, 2006). The search for new pharmacologically active agents obtained by screening natural sources such as medicinal plants or their extracts has led to the discovery of many clinically useful drugs that play a major role in the treatment of human diseases. Scientists are in search of new photochemicals that could be developed as useful anti- microbials for treatment of infectious diseases (Samy and Gopalakrishnakone, 2010). The abundance of medicinal plants in nature and the traditional knowledge increase the understanding of the medicinal plants properties, safety and efficacy (Nascimento *et al.*, 2000; Obeidat *et al.*, 2012). Many plants have been used for many years in folk medicine due to several purposes. Standard methods are used to screen preliminary photochemistry and quantitative analysis of active compounds. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties (Dash *et al.,* 2011). The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. Medicinal plants have an almost maximum ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivates. Most of these are secondary metabolites, of which 12000 plant- derived agents have been isolated in the recent past (Morton, 1981). Many of these substances serve as plant defense mechanisms against invasion by micro-organisms, insects and herbivores. The useful major groups of antimicrobial phytochemicals can be divided into several categories that include alkaloids, flavones (flavonoids, flavonols, quinones), essential oils, lectins, polypeptides,

phenolics, polyphenols, tannins and terpenoids. The present study aimed to investigate the pharmacological study in *Punica granatum* and *Mimosa hamata* with their antimicrobial activity against microorganism.

Mimosa hamata wild (Vern. Jinjani; Mimosaceae), a much branched, armed shrub, is commonly distributed along the open sandy places, often gregarious and abundant throughout the arid zone of Rajasthan, Punjab, Central and South India. It contains an alkaloid Mimosine. Roots contain tannin, ash, calcium oxalate crystals and mimosin. A crude alcoholic extract of its aerial part shows antibacterial properties. Leaves contain ethyl gallate and gallic acid (Hussain *et al.,* 1979; Mehta *et al.,* 1988). The whole plant of *Mimosa hamata* is very useful for various pharmacological and biological activities. Mostly Root and leaves of *Mimosa hamata* are showed maximum pharmacological activity (Katewa and Galav, 2005; Jain *et al.,* 1997).

Pomegranate, (*Punica granatum* L.) a species of Punicaceae, is the reddish-purple fruit of the pomegranate tree. The main chemical constitutes of *Punica granatum* are punicalagin, ellagic acid, luteolin, quercetin, kaempferol, ellagitannins and anthocyanins (delphindin, cyaniding and pelargonidin). The fruit rind yielded ellagic acid. The rind of the pomegranated fruit, and the bark of its tree, is prescribed by herbalists to treat intestinal worms and against amoebae (Julie, 2008). They bark is used against tapeworm and is also useful as a vaginal douche in leucorrhoea. Ellagic acid, one of the constituents of pomegranate juice and seed oils are reported as acting against cancer of skin, pancreas, breast, prostate, colon, intestine, oesophagus, bladder, oral, leukaemia, liver and neuroblastoma. Generally, pomegranate possessed the best antioxidant activity, independent on the antioxidant test assayed and generally with significant linear correlation between phenolics concentration and antioxidant capacity (Elfalleh *et al.,* 2009). *Punica granatum* fruit rind is rich in antioxidant of polyphenolic class which includes tannins & flavonoids. Antioxidant activity has been proposed to play vital role in various pharmacological activities such as anti-aging, anti-inflammatory, and anti-activities (Devatkal *et al*., 2010). Leaves, roots, bark stem and rhizome of *Punica granatum* exhibited anti-diarrhea activity for which the antibacterial effect against *E. coli, Shigella sonnei, S. flexneri* and *Salmonella typhi* of the hydrolysable tannins may be responsible (Mathabe *et al.,* 2006) but little is known about antimicrobial activity of fruit bark of *Punica granatum*.

Objectives

The objectives of proposed work are:

1. Extraction of secondary metabolites (steroids, triterpenoids, alkaloids etc.) different parts of selected plants and further fractionation.
2. Screening of extracted and identified metabolites against microorganism.
3. Identification of extracted compounds with the help of different chromatographic techniques.

Review of Literature

Infectious diseases are disorders caused by pathogenic microorganisms like bacteria, viruses, fungi, protozoa and multicellular parasites. These diseases are also called as communicable or transmissible diseases since they can be transmitted from one person to another via a vector or replicating agent. Infectious diseases account for about half of the deaths in tropical countries. Bacterial and fungal diseases are a type of infectious diseases caused by pathogenic bacteria and fungi (Solanki, 2010). Our world is endowed with a rich wealth of medicinal plants and man cannot survive on this earth for long healthy life without the plant kingdom because the plant products and their active constituents played an important role in maintaining perfect health.

Herbal medicine is the world's most ancient form of medicine as it is evident from the fact that every ancient civilization used plants for healing and many cultures, herbal knowledge was said to have been handed down from the god. Plants have always been the principal form of medicine in India and presently they are becoming popular throughout the world, as people strive to stay healthy in the face of chronic stress and pollution and to treat illness with medicines that work in count with the body's own defense (Perumalsamy *et al.*, 1998). Because of increasing resistance to antibiotics of many bacteria, plant extracts are of new interest as antiseptics and antimicrobial agents and also used for the treatment of skin disorders for centuries (Alipour and Khanmohammadi, 2011).

Secondary metabolites are a wide range of compounds from different metabolite families that can be highly inducible in response to stresses. These compounds are not essential for cell structure and maintenance of life but are often involved in plant protection against biotic and abiotic stresses (Weisshaar and Jenkins, 1998; Hattenschwiler and Vitousek, 2000). Some secondary metabolite families such as carotenoids and flavonoids are also involved in cell pigmentation in flower and seed, which attract pollinators and seed dispersers. Therefore, they are involved in plant reproduction (Winkel, 2001).

Moreover, plant secondary metabolites present chemical and pharmaceutical properties interesting for human health (Raskin *et al.,* 2002; Reddy *et al.,* 2003). Compounds belonging to the terpenoids, alkaloids and flavonoids are currently used as drugs or as dietary supplements to cure or prevent various diseases (Raskin *et al.,* 2002) and in particular some of

these compounds seem to be efficient in preventing and inhibiting various types of cancer (Watson *et al.*, 2001; Reddy *et al.*, 2003).

Seed powder of *Mimosa hamata* boiled in buffalo milk is given as a tonic in general weakness and also sexual weakness in males. Fresh leaf extract is applied to check bleeding from the wound and ulcer (Katewa and Galav, 2005). *M. hamata* synthesizes useful bioactive metabolites and a potential source of natural antioxidant (Jain *et al.*, 2009). *Mimosa pudica* is the herb that shows sensation on touch. It has been identified as Lajjalu in Ayurveda and has been found to have antiasthmatic, aphrodisiac, analgesic, anti-inflammatory and antidepressant activities. The antimicrobial activity of *Mimosa pudica* have been studied using disk diffusion method and the activity were tested against *Straphylococcus aureus, E. coli, P. aeruginosa* and *Candida albicans* (Kaur *et al.*, 2011). The hypolipidemic activity of *Mimosa pudica* extract has been studied on hyperlipidemic rats. Hyperlipidemia occurred due to enhancement in the levels of Cholesterols, Triglycerides, LDL and VLDL in rats (Sowmya and Ananthi, 2011).

Pomegranate, (*Punica granatum* L.) a species of Punicaceae, is the reddish-purple fruit of the pomegranate tree. In Ayurvedic medicine, the plant, described under its Sanskrit name "dalima" (fruit), is considered as a "blood tonic" and used to cure parasitic infections (Jurenka, 2008). The decoction of the root was found beneficial in fevers and chronic debility due to malaria. Moreover, the fruit rind powder was found to possess immunomodulatory properties (Gracious Ross *et al.*, 2001). Recently, studies have shown that pomegranate has many potential effects including: antiviral, immune modulation, vermifuge, stimulant, refrigerant, astringent, stomachic, styptic, laxative, diuretic and anthelmintic. Moreover, it serves to decrease the adverse effects of cardiovascular diseases, diabetes, diarrhea, dysentery, asthma, bronchitis, cough, bleeding disorders, fever, inflammation, acquired immune deficiency syndrome, dyspepsia, ulcers, bruises, sores, mouth lesions,skin lesions, malaria, prostate cancer, atherosclerosis, hypertension, periodontal diseases, hyperlipidemia, denture stomatitis, male infertility, vaginitis, erectile dysfunction, alzheimer, obesity and infant brain ischemia. Pomegranate is an amazing source of cyaniding, delphinidin (both are anthocyanidins), caffeic acid, chlorogenic acid (both are phenolic acids), gallic acid, ellagic acid (tannic acids), luteolin, quercetin (flavones), kaempferol (a flavonol), naringenin (a flavanone) as well as 17

alphaestradiol, estrone, estriol, testosterone, betasistosterol, coumesterol, gamma-tocopherol, punicie acid, campesterol and stigmasterol in its juice, peels and seed oil that are chemopreventive and therapeutic potentials of this plant (Lansky *et al.,* 2007; Kim *et al.,* 2002).

The extract of the leaves of *Punica granatum* abundant with tannins has been demonstrated to be a good gastric protective agent. It can increase the activity of pepsin, improve the secretion of bile, enhance the intestine peristallis, inhibit the secretion of gastric acid and decrease the incidence of gastric ulcer (Li *et al.,* 2003).

Anti-microbial properties of phenolic compounds active against pathogenic bacteria and fungi exhibited different sensitivities towards phenolics. These properties can be utilized in functional food development and for food preservation. Phenols are toxic to mico-organisms because of the sites and numbers of hydroxyl groups on the phenol groups, which is all related to their relative toxicity of micro-organism. There is evidence that highly oxidized phenols possess more inhibitory action (Puupponen *et al.*, 2001).

Methodology

1. *Punica granatum* fruit bark will be collected from local market of Jaipur and *Mimosa hamata* plant will be collected from Sariska National Park Alwar. Identification of plants will be carried out with the help of Department of Botany, Rajasthan University, Jaipur, Rajasthan.

2. *Mimosa hamata* whole plant and *Punica granatum* fruit bark will be dried. The fine cured powder will be used as herbal drug. Weighed amount of dried powdered of *Mimosa hamata* whole plant and *Punica granatum* fruit bark will be extracted to exhaustion in a Soxhlet apparatus.

3. 'Steroids will be extracted from crude plant materials by following the method of Tomita *et al.* (1970).

4. Triterpenoid and fatty acid derivatives will be isolated using standard protocol of Veliky and Latta (1974).

5. Alkaloid will be isolated using protocol of Wysocka and Przybyl (1994).

6. Total phenol content of the plant extract was estimated by Folin Ciocalteau method (Bray and Thorpe, 1954).

7. Identification of extracted compounds will be done with the help of chromatographic techniques.

8. Quantitative estimation of steroids, triterpenoids, alkaloids etc. will be carried out using well established methods.

9. Test microorganism: *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus, Staphylococcus epidermidis, and Klebsiella pneumoniae* bacterial and *Aspergillus niger, Aspergillus flavus*, *Trichoderma* sp., *Fusarium oxysporum* fungal samples will be selected for antimicrobial activity. All the bacterial and fungal species will be maintained on nutrient agar media and potato dextrose agar media.

10. Screening of extracted metabolites against microorganism will be done by ‘Disc Diffusion Assay’ (Bauer *et al.*, 1966).

11. Data will be tabulated and analyzed statistically.

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